

AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION

On page 3, line 12, please replace the original paragraph with the following amended paragraph:

-- Fig. 1A shows the result of mammalian two-hybrid assay; and Fig. 1B shows amino acid sequences of mouse (SEQ ID NO: 3) and human T2BPs (SEQ ID NO: 1) in comparison. --

On page 12, line 21, please replace the original paragraph with the following amended paragraph:

-- The assay was performed by using known method (15), that is, at first the assay samples were prepared by two-step PCR as described below using T2BP and TRAF2 cDNAs (contained in a mouse thymus-derived cDNA library from 3-day old mouse and in adult mouse testis-derived cDNA library, respectively) as templates, regions comprising their protein coding regions were amplified by PCR (first step) using the tagged gene-specific primers (forward primer T2BP, gaaggagccgccaccatgtccaccttggaagacg (SEQ ID NO: 5); forward primer TRAF2, gaaggagccgccaccatggctgcagccagtgt (SEQ ID NO: 6); designed by using known software for protein region prediction) and the primer for the vector sequence (reverse primer P8; agcggataacaatttcacacaggaaa) (SEQ ID NO: 7). DNA fragments for SV40 poly-A signal and for human cytomegalo virus (CMV) immediate early promoter followed by Gal4 DNA-binding domain or herpes virus VP16 transcriptional activation domain were also amplified (sequences of forward and reverse primers and templates used herein were respectively as follows; DNA fragment of SV40 poly-A signal, gtttcctgtgtgaaattgttatccgctgcagacatgataagatacattg (forward) (SEQ ID NO: 8),

agcaagttcagcctggtaagatccttatcgattttaccac (reverse) (SEQ ID NO: 9), pG5*luc* (Promega); Gal4 fragment, ccaatatgaccgccatgttggc (forward) (SEQ ID NO: 10), catggtggcggctccttcggcgatacagtcactg (reverse) (SEQ ID NO: 11), pBIND (Promega); VP16 fragment, ccaatatgaccgccatgttggc (forward) (SEQ ID NO: 12), catggtggcggctccttcaagtcgacggatccctggc (reverse) (SEQ ID NO: 13), pACT (Promega)). In the second PCR, the first PCR product, the SV40 poly-A signal fragment and the Gal4- or VP16- fragment were ligated so as to design that the PCR product is expressed as a fusion protein with the Gal4- or VP16-domain. The sequences of the primers used in the second PCR were, gccatgttggcattgatttgac (forward) (SEQ ID NO: 14) and agcaagttcagcctggtaag (reverse) (SEQ ID NO: 15). The PCR products (0.13 μ l) were transfected together with 20 ng of reporter plasmid pG5*luc* into 2.2×10^4 of CHO-K1 cells using the transfection reagent LF2000 (Invitrogen). After 20 h of incubation, the luciferase reporter activity was measured with the Steady-Glo (trade name) Luciferase Assay System (Promega). --

On page 13, line 23, please replace the original paragraph with the following amended paragraph:

--The regions comprising the protein coding regions of T2BP and TRAF2 cDNAs were amplified by PCR using the gene specific primers (forward primer sequence for T2BP; gacgcgtcgaccatgtccaccttgaagacg (SEQ ID NO: 16), forward primer sequence for TRAF2; gacgcgtcgaccatggctgcagccagtgt (SEQ ID NO: 17). Reverse primer was designed for vector region, the sequence was, ccggttaagcggccgcagcggataacaatttcacacaggaaac.) (SEQ ID NO: 18) and then sub-cloned into the expression vectors pCMV-HA and pCMV-Myc (both produced by Clontech), respectively.

293T cells (1×10^6) were transfected with 2.5 μ g of the expression vectors for HA-T2BP and Myc-T2BP using the a transfection reagent LF2000 (trade name). HA and Myc denote the names of the tag sequences recognized by antibodies. After 24 h of incubation, cells were harvested and lysed by TNE buffer consisting of 10 mM of Tris-HCl (pH 7.8), 1% NP40 (trade name), 0.15 M NaCl, 1 mM EDTA, 1 mM PMSF and 10 μ g/ml leupeptin (PEPTIDE INSTITUTE Inc.). After centrifugation at 10,000 x g for 15 min, the supernatants were isolated and immunoprecipitated with 5 μ g anti-HA antibody (Santa Cruz). Detection of the co-precipitated Myc-TRAF2 was performed by Western blot analysis. Samples in Laemmuli sample buffer were boiled for 5 min and subjected to 12.5% SDS-PAGE, and proteins were transferred onto a Hybond-ECL membrane (Amersham). Western blotting was performed according to standard procedures by incubating with an anti-Myc antibody (Clontech) for 1 h and HRP-conjugated anti-mouse IgG (Amersham) for 1 h followed by washing steps. Detection of the signal was performed using the ECL system (Amersham) and X-ray film (Kodak). The supernatants described above were also subjected to direct Western blot analysis to confirm the expression of HA-T2BP and Myc-TRAF2 using the primary and secondary antibodies shown above. - -